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**PATENT APPLICATION**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

HIBINO

Application Serial No. 09/444,388

Filing Date: November 22, 1999

For: PROCESS FOR OBTAINING PLANT DNA FRAGMENTS AND USE THEREOF

**AMENDMENT UNDER 37 CFR §1.111**

Commissioner for Patents  
Washington, D.C. 20231

August 13, 2001

Sir:

This Amendment under 37 CFR §1.111 is being filed in response to the Office Action dated April 11, 2001, making the response due on or before August 11, 2001 with a Petition for Extension of Time for one month, attached hereto.

Please amend the application as follows.

**IN THE CLAIMS:**

Please delete claims 1-5 without prejudice or disclaimer.

Please add new claims 8-16 as follows:

--8. A method for identifying a breeding marker for polymorphic plants,

comprising the steps of:

- a) selecting two sibling individuals of a plant having different phenotypes;
- b) obtaining genomic DNA from the individuals;



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- c) isolating and selecting DNA fragments by a genome subtraction method using the genomic DNA from the individuals;
  - d) providing an RNA-derived labeled probes wherein the probe is a labeled cDNA of at least one mRNA obtained from the individuals, and the cDNA is selected and amplified by oligonucleotide primers in a polymerase chain reaction, wherein the primers are designed to hybridize to the mRNA for a plant gene<sup>11</sup> related to the breeding marker;
  - e) fractionating the DNA fragments and screening the DNA fragments with the RNA-derived labeled probe; and
  - f) detecting binding between the DNA fragments and the RNA-derived labeled probe.

Accepted

9. The method of claim 8, wherein the plant is a forest tree.

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10. The method of claim 9, wherein the forest tree is Acacia.

11. The method of claim 10, wherein the Acacia is a species *Acacia auricaliformis*.

12. The method of claim 8, wherein the genome subtraction method is representation difference analysis method.

13. The method of claim 8, wherein the labeled cDNA is labeled with digoxigenin.

14. The method of claim 8, wherein the oligonucleotide primers comprise the sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

15. The method of claim 8, wherein the DNA fragments are fractionated by acrylamide gel electrophoresis.

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16. The method of claim 8, wherein for step (e), screening is further carried out for each individual, with DNA fragments isolated by genome subtraction from the same individual.--

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### REMARKS

Claims 1-7 are all the pending claims. Claims 6-7 have been withdrawn as being directed to non-elected subject matter. By this amendment claim 1-5 have been cancelled and new claims 8-16 have been added hereby.

New claims 8-16 find support in the original specification as follows:

- Claim 8 finds general support, inter alia, in canceled claim 1 and specific support in Example 1 starting on page 8, line 27 and continuing to page 11;
- Claim 9 finds support on page 1 at line 29 and page 2, line 35;
- Claim 10 finds support in Figures 1-3 and page 5 at line 37;
- Claim 11 finds support on page 8 at line 33;

- Claim 12 finds support on page 6 at lines 7-8 and in Example 1 (page 9, lines 1-3);
- Claim 13 finds support on page 6 at lines 14-26;
- Claim 14 finds support on page 9 at lines 14-28;
- Claim 15 finds support on page 5, line 8 and in Example 1 (page 9, lines 30-31); and
- Claim 16 finds support on page 10, lines 9-12.

No new matter has been added, and consideration and entry of the new claims is requested.

#### **I. Response to Formal Matters**

According to the Examiner, the original application as filed did not contain an abstract.

The abstract appears to have been lost or misplaced at the USPTO. As proof of filing, Applicants are enclosing a copy of the application transmittal letter of November 22, 1999 indicating that 14 pages of the original specification were filed, and that page 14 contained the abstract. Applicants are also enclosing a paper copy of the originally filed abstract which now satisfies the requirement for a complete specification.

#### **II. Response to Restriction Requirement**

A provisional election was made by telephone on January 18, 2001, to prosecute Claims 1-5 (drawn to a method for obtaining a plant DNA fragment and to a gene

encoding the fragment) of Group I without traverse. Accordingly, Applicants affirm their election herewith.

### **III. Response to the Rejection of Claims 2-4 under 35 U.S.C. §101**

Claims 2-4 are rejected under 35 U.S.C. §101 for being directed to non-statutory subject matter.

Claims 2-4 have been cancelled, and therefore, the examiner's rejection has been rendered moot. New claims 8-16 are fully supported and enabled by the specification, and are directed to statutory subject matter under 35 U.S.C. §101.

### **IV. Response to the Rejection of Claims 1-5 under 35 U.S.C. § 112, first paragraph**

A. Claims 1-5 are rejected under 35 U.S.C. 112, first paragraph, for lack of enablement.

In the Office Action, the Examiner states that the claims are broadly drawn to a process for obtaining any plant DNA fragment by digesting DNA from any plant, subjecting the fragments to subtractive hybridization to obtain polymorphic fragments and screening the fragments to obtain a desired fragment. The claims are further drawn to any fragment obtained by this method, including any "desired" gene and any DNA with promoter activity.

The specification only teaches a general method of obtaining DNA fragments from plants using genome subtraction, and does not teach whether these fragments are the "desired" fragments as encompassed by the claimed invention.

Applicants traverse for the following reasons.

Initially, Applicants submit that the Examiner's rejection is moot in view of canceled claims 1-5.

The specification teaches the RDA method for genome subtraction (see p. 8, line 31) of DNA obtained from two sibling plant leaves of *Acacia auricaliformis*. The specification teaches that positive DNA fragments were subcloned and analyzed and that 6 DNA fragments were obtained (see p. 10, lines 20-22, and FIG 1). The specification teaches that FIG 1 shows the results of genome subtraction and hybridization by an expression probe for acacia, and that the circles indicate DNA fragments selected by subtraction that were judged to be complementary with the experimental probe (see p. 11, lines 12). New claims 8-16 recite these aspects of the invention, making the claimed subject matter compliant under the first paragraph of §112. Accordingly, the Examiner's rejection is rendered moot.

**V. Response to the Rejection of Claim 1 under 35 U.S.C. 112, second paragraph**

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

In the Office Action, the Examiner states that Claim 1 is indefinite in the recitation of "RNA derived probe" as it is unclear whether the term refers to a probe that hybridizes to cDNA or to a probe that has been altered in some way.

Applicants traverse for the following reasons.

Initially, Applicants submit that the Examiner's rejection is moot in view of canceled claim 1.

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The "RNA derived probe" is disclosed as being a complementary DNA (cDNA) of an RNA wherein the cDNA optionally contains a label. New claims 8-16 recite this aspect of the invention and are written in definite language, therefore the claims are fully compliant under the second paragraph of §112.

**VI. Response to the Rejection of Claim 1 under 35 U.S.C. §§102(b)/103(a)**

Claim 1 is rejected under 35 U.S.C. 102(b) as anticipated by Wigler et al (US Patent 5,436,142) or, in the alternative, under 35 U.S.C. 103(a) as obvious over Wigler et al (US Patent 5,436,142).

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In the Office Action, the Examiner states that Wigler discloses representational different analysis (RDA) for generation of DNA fragments that can be used as probes to identify polymorphic sites (col. 5), and that the method is applicable to plants. In example 2, Wigler specifically teaches analysis of DNA from two individuals resulting in the detection of a small number of differences between two nearly identical genomes.

Applicants traverse for the following reasons.

Initially, Applicants submit that the Examiner's rejection is moot in view of canceled claim 1.

New claims 8-16 are directed to the RDA method for detecting differential Acacia gene expression.

Wigler may teach a method for isolating genes from the genus of plants, but a genus does not anticipate or render obvious a species. The present claimed method is drawn to one for characterizing genes from Acacia or Acacia auricaliformis, and specifically avoids the teachings of Wigler.

Additionally, the claimed method essentially involves a two step approach, first involving subtraction of genomic DNA followed by probing of the DNA with cDNA's obtained from the mRNA of sample tissues from the same individual or forest tree source. Wigler is specifically silent with respect to these claimed elements, and therefore Wigler is not an effective reference under §102(b).

The claims do not read on, and therefore, are not anticipated or rendered obvious by Wigler '142. Withdrawal of the rejection is deemed proper.

#### **VII. Response to the Rejection of Claims 1-5 under 35 U.S.C. §102(b)**

Claims 1-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Phillips et al (Plant Molecular Biology, vol. 24, pp 603-615, 1994).

In the Office Action, the Examiner states that Phillips teaches a subtraction cloning scheme for *Arabidopsis thaliana*, which resulted in the isolation of differentially regulated cDNA (see abstract). Phillips teaches that the probes for differential hybridization were generated from single strand cDNA (p. 607, co. 1) and that the technique identified two genes in plants of the *Arabidopsis thaliana* strain (p. 613, col. 1, "Discussion").

Applicants traverse for the following reasons.

Initially, Applicants submit that the Examiner's rejection is moot in view of canceled claims 1-5.

Phillips teaches genes from *Arabidopsis thaliana*. New claims 8-16 avoid this reference because they are directed to a subtraction/hybridization method involving *Acacia* or *Acacia auriculiformis* plant genes. Since a species does not anticipate



another species (i.e., the Phillips method does not anticipate the claimed method, much less that the method can be applied to the isolation and characterization of genes from the instant claimed plant species.

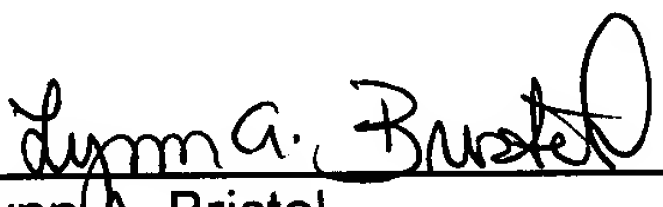
The instant claims directed to a method for screening Acacia genes do not read on, and therefore, are not anticipated by Phillips. Withdrawal of the rejection is deemed proper.

### CONCLUSION

Applicants respectfully submit that in view of the foregoing comments, that the claims are now in condition for allowance. Applicants request that the Examiner find the above-referenced application allowable, and that the application pass to issue.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,

  
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Enclosures: Copy of the Application transmittal letter of November 22, 1999 / Copy of the Abstract filed on November 22, 1999